Meeting Report

The Role of the Environment in Parkinson's Disease

Jay M. Gorrell,[1] Donato DiMonte,[2] and Doyle Graham[3]

[1]Department of Neurology, Henry Ford Hospital, Detroit, MI 48202 USA; [2]The Parkinson's Institute, Sunnyvale, CA 94089-1605 USA; [3]Department of Pathology, Vanderbilt University, Nashville, TN 37215 USA

Thirty leading scientists in the field of Parkinson's disease research attended a conference, "The Role of the Environment in Parkinson's Disease," 17-19 September 1995, sponsored and hosted by the National Institute of Environmental Health Sciences. Parkinson's disease investigators working in various basic and clinical disciplines presented and evaluated current scientific findings concerning the etiology of the disease and charted the most fruitful courses for future research. The role of the environment was highlighted, but considerable attention was given to pathological neurochemistry and genetic issues in the etiopathogenesis of Parkinson's disease.

The conference was opened by Annette Kirshner, program administrator at NIEHS and chair of the conference, who framed the context of the discussions to take place. Terri Damstra, acting deputy director of NIEHS, welcomed attendees to the institute and reaffirmed the importance of the work to NIEHS. She emphasized that in Parkinson's disease the potential roles of environmental exposure to one or more agents, genetic susceptibility to such exposures, and the factor of time or aging are likely to play roles in the etiology of the neurodegenerative process.

Formal presentations were divided into three sessions: environmental and genetic risk factors (Doyle Graham, chair), neurotoxins and mechanisms of neuronal injury (Jay M. Gorell, chair), and biological markers of Parkinson's disease (Donato DiMonte, chair). Summaries of each of these sessions follow.

Environmental and Genetic Risk Factors

The relative roles of environmental, endogenous neurochemical and genetic factors in the etiology of Parkinson's disease is currently unclear. For example, the prevalence of Parkinson's disease in a community could be due to a differential distribution of a hypothetical environmental toxicant or be more frequent where a heritable defect is common. Twin studies in the 1980s seemed to discount a significant genetic role in Parkinson's disease when the degree of concordance between monozygotic and dizygotic pairs was found to be similar. An environmental cause of the disease was favored when it was shown that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could enter the brain and be metabolized to 1-methyl-4-phenylpyridinium ion (MPP^+), which could specifically destroy nigral neurons to produce a human condition that closely mimicked Parkinson's disease. Moreover, the fact that the amyotrophic lateral sclerosis/Parkinson's disease/dementia complex could develop in Guamanians decades after their migration from the island strengthened the idea that a long latent period after exposure to an environmental toxicant could be present before a degenerative disorder was fully manifest.

The potential role of genetics in Parkinson's disease was raised again when a number of families with parkinsonism in several generations was reported. Few affected individuals have been autopsied thus far, but typical Lewy body histopathology has been found. Some investigators believe that many more familial Parkinson's disease cases will be found, but, clearly, a biomarker of genetic risk would be optimal for case identification. Allelic association studies and DNA linkage analysis have not tied the occurrence of Parkinson's disease to various candidate genes so far, though a systematic search of the human genome is underway.

As work on genetics proceeds, much productive research continues to identify endogenous neurochemical abnormalities, to study the mechanisms of action of neurotoxins, and to characterize environmental risk factors that may either initiate or perpetuate Parkinson's disease. In the epidemiologic literature, Parkinson's disease has been consistently shown to increase with either advancing age or the passage of time and to be slightly more prevalent in males. Moreover, nearly all studies report an inverse (i.e., protective) association of Parkinson's disease with smoking, though the mechanism by which this influences risk is unclear. Some studies have suggested a decreased risk of Parkinson's disease with a higher intake of antioxidant vitamins, and many others have found an increased risk of Parkinson's disease among those who have lived in rural environments, farmed, or been exposed to pesticides or to well water.

Two population-based case–control studies have been conducted. Semchuk and colleagues, at the Universities of Calgary and Saskatchewan, found that a family history of Parkinson's disease was the strongest predictor of Parkinson's disease risk, followed by a history of head injury severe enough to require medical attention, and then by a history of occupational exposure to herbicides. Gorell and colleagues, at Henry Ford Hospital in Detroit, Michigan found an increased risk of Parkinson's disease with more than 10 years of occupational exposure to copper, manganese, or lead, but no increased risk with iron, zinc, or mercury exposure. Gorell et al. also found an increased risk of Parkinson's disease with occupational exposure to either herbicides or insecticides.

There is active investigation of potential risk of Parkinson's disease in the systemic metabolism of xenobiotics, operating either via cytochrome P450 enzymes (e.g., CYP2D6 in the detoxication of MPTP and isoquinolines) or through impaired sulfation and sulfoxidation. Finally, there is an ongoing, large study of identical twins, examining their concordance for Parkinson's disease, which could help define the relative roles of genetic and nongenetic factors.

Ultimately, there may be a varying mixture of factors that produce Parkinson's disease in different human populations. For example, in families showing an autosomal dominant pattern of Parkinson's disease transmission, genetic factors may dominate. Among other populations (currently believed to account for the majority of cases) a variety of environmentally acquired neurotoxic exposure(s) may interact under the oxidatively stressed conditions in the Parkinson's disease substantia nigra (SN) to produce the disease. More than one agent (e.g., xenobiotics, metals) may produce Parkinson's disease in sequential stages. Finally, various nongenetic and genetic mechanisms may be linked if the genome is damaged by environmental or endogenous toxins. However, any model proposed to explain the occurrence of Parkinson's disease must account for the selective vulnerability of nigral neurons, the susceptibility of particular individuals, and disease progression.

Neurotoxins and Mechanisms of Neuronal Injury

The chemical pathology of neuromelanin and oxidative stress in Parkinson's disease

Address correpondence to A.G. Kirshner, MD 3-03, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, NC USA.

may be pivotal in nigral neuronal death. Neuromelanin, made from orthoquinones derived from catecholamine metabolism, forms during the gradual depopulation of pigmented catecholaminergic neurons in the central nervous system in Parkinson's disease. The chemical milieu surrounding neuromelanin is characterized by transition metal-catalyzed redox cycling between semiquinone and catecholamine compounds, as well as by the formation of hydrogen peroxide from the catabolism of catecholamines by either the action of monoamine oxidase (MAO) or by their autoxidation. Cellular factors that increase SN oxidative reactions, such as potential activation of xenobiotics by MAO (as with MPTP), interference with mitochondrial oxidative phosphorylation (e.g., by inhibition of complex I), or action by excitotoxins, can exacerbate local oxidative stress.

There is an apparently Parkinson's disease-specific 35–40% decrease in SN mitochondrial complex I function, the same defect seen with MPP^+ action. This deficiency could generate superoxide and other free radicals that may contribute to neuronal damage in the SN. However, it is unclear whether the SN complex I deficiency in Parkinson's disease is due to genetic damage, exogenous or endogenous factors, or both. A possible genetic defect may be due to alterations in nuclear and/or mitochondrial DNA, as several complex I subunits are encoded by mitochondrial DNA.

Pharmacological study of model systems such as P19 neuroglial cultures can shed further light on neurochemical changes in oxidatively stressed brain. For example, the cytotoxicity of lipophilic dopamine congeners in this system is enhanced by manganese coincubation and is associated with cross linking of neurofilament protein into high molecular weight aggregates. Moreover, redox-cycling catechols induce lipid peroxidation that generates E-4-hydroxy-2-nonenal, a protein crosslinking agent. These results suggest that lipid peroxidation, and perhaps Lewy body formation with subsequent neurodegeneration, may be related to catechol oxidation.

Iron levels are increased approximately 35% in the Parkinson's disease SN zona compacta, and laser microprobe and X-ray microanalysis studies show increased iron (and aluminum) in SN neuromelanin in such patients. Moreover, iron infusion into SN in animals can cause both acute and progressive neuronal loss. The importance of these findings may lie in the fact that iron, if liberated from neuromelanin (or ferritin) stores, can greatly potentiate the formation of highly cytotoxic hydroxyl radicals from hydrogen peroxide, which is produced from dopamine metabolism. This process may be more likely in the Parkinson's disease brain, in which a deficiency of reduced glutathione (GSH), the major defense against hydroxyl radical formation, has been found.

In a monkey model of manganism produced by intravenous infusion of $MnCl_2$, the metal accumulated in putamen, with a subsequent massive periventricular accumulation of iron and an increase in aluminum in globus pallidus. These findings raise the possibility that environmental exposure to manganese may also reflect exposure to iron and aluminum. Because the liver is the major organ involved in the clearance of manganese, liver failure could predispose an individual to manganese-induced neurotoxicity. Finally, though aluminum does not promote lipid peroxidation, it greatly potentiates the ability of iron to do so, raising the possibility that all three metals may participate in the pathogenesis of Parkinson's disease.

Several classes of neurotoxins have been studied as potential nigral toxins, with varying results. For example, some β-carboline compounds are structurally similar to MPP^+ and have been shown to inhibit mitochondrial complex I. Though substances studied so far do not show dopaminergic cytotoxic specificity in rat primary mesencephalic cultures, 2,9-di-*N*-methyl β-carbolinium derivatives are detectable in the cerebrospinal fluid of some Parkinson's disease patients, and such compounds are eight-fold more concentrated in Parkinson's disease SN than in cerebral cortex. The organochlorine pesticide dieldrin was present in 6 of 20 Parkinson's disease brains in one study, though it was not more concentrated in Parkinson's disease samples, suggesting that it was not sufficient to cause Parkinson's disease.

Four models were considered in the identification of agents with potential SN toxicity: 1) utilization of the dopamine uptake system in nigrostriatal neurons to confer specificity (e.g., MPP^+); 2) interaction of toxins with dopamine (e.g., methamphetamine-induced striatal terminal damage); 3) utilization of iron in free radical generation and/or in the bioactivation of an environmental toxin (e.g., MPP^+ generation by iron-activated, MAO-independent means); and 4) potentiating effects of two agents at one or more sites (e.g., diethyldithiocarbamate plus L-dopa may act by inhibition of superoxide dismutase, increased bioactivation by MAO, decreased dopamine re-uptake, decreased mitochondrial oxidative function, and by glutamatergic excitotoxicity).

Biological Markers

Brainstem Lewy body formation is a neuropathological hallmark in the diagnosis of Parkinson's disease postmortem. However, little is known about its role in the pathogenesis of Parkinson's disease, nor is much known about the role of neuronal inclusions in MPTP-parkinsonism. The reasons for differences in the inclusions in these disorders is needed to clarify the pathogenesis of both Parkinson's disease and MPTP-parkinsonism.

Thus far, it has not been possible to find a specific biomarker of Parkinson's disease in accessible tissues of living patients. For example, there has been an extensive search for a mitochondrial complex I defect in peripheral tissues. However, the existence such a deficiency in skeletal muscle is in doubt. Moreover, the consistent decrease in complex I activity in Parkinson's disease platelets shows overlap with control values, and the extent of the deficiency is variable.

No significant associations have been found between superoxide dismutase-1 (SOD-1), Huntington (IT-15), ApoE4 and ApoE3, CYP2D6 alleles, or MAO-A haplotypes and Parkinson's disease. Analysis has failed to show genetic linkage in three Parkinson's disease families with glutathione peroxidase, tyrosine hydroxylase, amyloid precursor protein, SOD-1, CYP2D6, or chromosomal regions expressing choline acetyltransferase or brain-derived neurotrophic factor. Further studies are in progress to define a role for a mutant allele of CYP2D6 and to test the hypothesis that alterations in detoxification enzymes may be markers of risk factors for Parkinson's disease.

The value of cerebrospinal fluid data as a marker of the dopaminergic system in Parkinson's disease has been limited. A decrease in cerebrospinal fluid homovanillic acid (HVA) has been shown in Parkinson's disease in many, but not all, studies, and there is no correlation between the HVA level and either the duration or severity of the disease. There has been no follow-up of an earlier report suggesting that the 5-*S*-cysteinyl forms of dopamine, DOPAC and L-dopa, in cerebrospinal fluid may be indices of oxidative stress in basal ganglia.

Imaging studies offer promise in providing markers of Parkinson's disease. For example, positron emission tomography (PET) studies have shown a linear correlation between the extent of reduced 6-fluorodopa uptake in striatum and the numbers of SN neurons in subsequent autopsies in some Parkinson's disease patients. Higher resolution PET scanners can differentiate

caudate and putamen, and Parkinson's disease patients can be completely separated from control subjects. The two populations can be mostly resolved by imaging striatal binding of the dopamine antagonist raclopride. The single-photon emission tomography radioligand, [^{123}I]β-carbomethoxy-3-β(4-iodophenyl)-tropane, a cocaine analogue that binds to the dopamine transporter in striatum, has been used to show a direct relationship between diminished uptake and motor disability in Parkinson's disease patients. Finally, a new MRI method has been developed to assess iron accumulation at 3 Tesla in SN. The method relies on the calculation of $1/T_2'$ (R_2'), the relaxation rate due to local paramagnetic material. R_2' in the dark area of the SN on T_2 or T_2*-weighted images is significantly higher in Parkinson's disease patients, and does not overlap with control values, reflecting the higher iron content in Parkinson's disease SN postmortem. A direct correlation has been shown between the right/left asymmetry of SN R_2' versus the left/right asymmetry of simple reaction time in Parkinson's disease subjects, suggesting that SN iron accumulation is related to disease severity.

Conclusions

Areas of focus for future research were identified, which should be pursued by several NIH institutes. Epidemiological studies need to 1) identify exposures to particular toxins that can specifically damage SN or other basal ganglionic structures, 2) clarify the age-specific rates of disease to understand trends over time, 3) more specifically define risk factors being assessed, 4) examine whether a family history of other neurodegenerative diseases increases the risk for Parkinson's disease, 5) study Parkinson's disease subgroups (e.g., young, old, familial, and nonfamilial cases) to determine differential risk for acquiring the disease, and 6) bank specimens of blood and other tissues to link environmental exposures to markers of Parkinson's disease; i.e., of the disease itself, of mechanisms of biochemical damage, and of potential genetic risk. Finding biomarkers for Parkinson's disease would enhance the reliability of clinical diagnosis, helping to ensure the homogeneity of cases selected for clinical and epidemiologic studies; permit preclinical diagnosis, enabling full ascertainment of familial cases, identification of those at risk for selected environmental toxicants, and allow selection of individuals for trials of possibly neuroprotective drugs; potentially permit the screening of large populations; and possibly provide clues about the etiopathogenesis of the disease.

Understanding the mechanism(s) underlying brainstem Lewy body formation and its role in the neurochemical pathology of Parkinson's disease could elucidate the etiopathogenesis of the condition. Identifying early or initiating neurochemical events is critical, including factors conferring selective vulnerability or protection to nigral neurons (e.g., chemical characteristics of toxins; the role of glial cells in the bioactivation of toxins or in providing neuroprotection; neurotrophic factors). The role of bioaccumulation of toxicants (e.g., metals, glutamate, pesticides, infectious agents, others), with slow release over time, should be investigated. The development of models of chronic *in vivo* neurotoxicity that more closely resemble the progressive nature of Parkinson's disease should be a priority.

Continued search for one or more genes in Parkinson's disease with linkage to brainstem Lewy body pathology is an important area of investigation. More autopsies are needed in large kindreds to establish the pathological basis for their clinical condition. Results of the ongoing study of a large number of identical twins with one Parkinson's disease twin could clarify a potential genetic role in Parkinson's disease.

The potential interaction between the environment, pathological neurochemistry, anatomy, and genetic factors was seen as the most fruitful overall research direction to unravel the etiology and pathogenesis of Parkinson's disease. Sharing insights in the basic and clinical neurosciences is the best vehicle for maximally using diverse scientific talent. Interdisciplinary research should be encouraged by NIH institutes.

Co-sponsors of the Workshop on the Role of the Environment in Parkinson's Disease

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Organizing Committee

Annette G. Kirshner
NIEHS
Research Triangle Park, North Carolina

Jay M. Gorell
Department of Neurology
Henry Ford Hospital
Detroit, Michigan

Donato DiMonte
The Parkinson's Institute
Sunnyvale, California

Doyle Graham
Department of Pathology
Vanderbilt University
Nashville, Tennessee

Thomas Montine
Department of Pathology/Toxicology
Vanderbilt University
Nashville, Tennessee